Cain and Abel reloaded? Kin recognition and male–male aggression in three-spined sticklebacks *Gasterosteus aculeatus* L.

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The influence of relatedness on male–male aggression was tested in three-spined sticklebacks *Gasterosteus aculeatus*. The intensity of aggression against brothers and non-kin males did not differ significantly, indicating that kin recognition plays at most a minor role in aggressive interactions between male *G. aculeatus*. © 2009 The Authors

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The ability to discriminate between related and unrelated individuals has been demonstrated for a whole range of taxa, from social microbes *Dictyostelium purpureum* (Mehdiabadi *et al*., 2006) to chimpanzees *Pan troglodytes* (Vokey *et al*., 2004). The mechanisms of kin recognition are well studied (Mateo, 2004), whereas its function often remains unclear. It is probably understood best in mate choice, where individuals that are able to recognize relatives may avoid the negative effects of inbreeding (Charlesworth & Charlesworth, 1987; Waldman & McKinnon, 1993; Frommen & Bakker, 2006) although in some cases positive effects have been shown (Thünken *et al*., 2007). Also during shoaling decisions, preferences for groups composed of related individuals have been demonstrated frequently (Ward & Hart, 2003). Shoaling with relatives may be beneficial because altruistic behaviour towards kin is thought to increase an individual's indirect fitness (Hamilton, 1964). Such a benefit was for instance suggested in studies of Atlantic salmon *Salmo salar* L. and rainbow trout *Oncorhynchus mykiss* (Walbaum), in which the mean frequency of aggressive interactions in shoals of related individuals was lower than in non-kin groups (Brown & Brown, 1993). Additionally, the survival rate in a group composed of related pike *Esox lucius* L. was higher than in a group of unrelated individuals (Bry & Gillet, 1980). In zebrafish *Danio rerio* (Hamilton) fry in kin groups grew faster, although there was no reduction in aggressive behaviour (Gerlach *et al*., 2007). Altruistic behaviour towards relatives should also be beneficial in intrasexual aggressive behaviour like territorial defence. In the fire salamander *Salamandra infraimmaculata*, for example, it has been shown that aggression and associated

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injuries decreased as genetic similarity increased (Markmann *et al.*, 2009). In contrast, in a study on house sparrows *Passer domesticus*, no reduced aggression towards kin was found (Tóth *et al.*, 2009). Male–male aggression, however, is underrepresented in studies of kin recognition.

In this study, three-spined sticklebacks *Gasterosteus aculeatus* L. were used as model species. As their ability to recognize kin has been demonstrated in different contexts, they are an excellent system to study the influence of relatedness on aggressive interactions. During shoaling decisions individuals of different age classes preferred the group composed of related individuals (FitzGerald & Morrissette, 1992; Frommen & Bakker, 2004; Frommen *et al.*, 2007a), while they avoided their relatives during mate choice (Frommen & Bakker, 2006). Furthermore, clutches containing unrelated eggs were cannibalized more often by males (Frommen *et al.*, 2007b). It is unknown, however, whether males use this ability in the context of intrasexual aggression.

During the breeding cycle, male *G. aculeatus* hold territories in shallow parts of the habitat and build a nest composed of algae, in which gravid females spawn. As good territories are scarce, males vigorously fight for them. Territories are often located close to each other so that sneaked fertilizations and egg stealing are a common phenomenon in this species (Goldschmidt *et al.*, 1992; Jamieson & Colgan, 1992; Largiadèr *et al.*, 2001; Zbinden *et al.*, 2003, 2004). Furthermore, neighbouring males often compete for females (Dzieweczynski & Rowland, 2004). Males thus have to remain aggressive after establishing their territory. After spawning, the male takes care of the developing embryos until they hatch, by fanning and cleaning the clutch and defending the territory against rival males (Wootton, 1984) or brood-raiding females (Whoriskey & FitzGerald, 1985; de Fraipont *et al.*, 1992). The level of male aggression in intrasexual encounters is influenced by several factors such as breeding colouration (Bakker & Sevenster, 1983; Rowland *et al.*, 1995; Rick & Bakker, 2008), body size (Larson, 1976; Rowland, 1989; although Sargent & Gebler, 1980 and van den Assem, 1967, have shown the opposite), experiential effects, value of the resource or distance to the nest (Bakker, 1994a; Rowland, 1994). Finally, as *G. aculeatus* is a colonizing species (Bell & Foster, 1994), populations are often founded by only a small number of individuals, leading to a rather small gene pool (Heckel *et al.*, 2002; Raeymaekers *et al.*, 2005). Consequently, at least in small populations, there is a real chance to meet related individuals during the reproductive season (Frommen *et al.*, 2008). In addition, non-reproductive *G. aculeatus* from the population used in this study prefer to shoal with familiar as well as with unfamiliar kin (Frommen *et al.*, 2007a). Hence, it is conceivable that related males arrive at the same time in a potential breeding habitat. It is unknown, however, whether related individuals show reduced levels of aggression among each other than unrelated individuals.

The present study aimed at testing whether territorial *G. aculeatus* males are less aggressive towards their brothers. As kin recognition in reproductively active *G. aculeatus* is known to be triggered by olfactory cues (Mehlis *et al.*, 2008), the experiments were conducted using water conditioned by a familiar brother and an unfamiliar unrelated male in combination with two identical computer animations as visual stimuli (Künzler & Bakker, 1998; Mehlis *et al.*, 2008).

Fish from an anadromous, genetically heterogeneous population (Heckel *et al.*, 2002) were caught during their spring migration in April on the Island of Texel (53° 05′ N; 4° 50′ E), the Netherlands. Fish used in the experiment were bred in the

laboratory from wild-caught parents (sibling groups) or from F1 or F2 offspring (sibling groups that had been inbred for one or two generations). Because preferences did not significantly differ between out and inbred individuals (Mann–Whitney U-test, all $P > 0.05$, the data for out and inbred individuals were combined. Detailed rearing conditions are described in Mehlis *et al.* (2008). Two months before the start of the experiments, the light regime was set to summer conditions (16L:8D, temperature 17° C, range $\pm 1°$ C), stimulating males to become sexual active.

Experimental trials were conducted between November and December 2006. Aggressive behaviour was tested in a glass tank measuring $45 \times 40 \times 30$ cm. The tank was filled with 1 day-old tap water and lit by a 36 W fluorescent lamp placed 91 cm above the bottom of the tank. It was visually isolated by placing grey plastic plates on all sides and tightening a black curtain around the tank. Two small windows (7.5×30 cm) on opposite sides of the tank allowed the test male to see two identical computer animations of dull-coloured, sexually active males (Mehlis *et al.*, 2008). The computer animations were presented on two identical monitors (Sony, Trinitron, Multiscan 200 Ps, 1024×768 Pixel, 85 Hz; www.sony.com) that were placed at a distance of 2.5 cm from each side of the tank (Mehlis *et al.*, 2008). The computer-animated male showed reproductive behaviour like fanning near the nest and zig-zagging towards the test male, which is also part of male–male aggression (Rowland, 1988; Bakker, 1994*b*).

In front of the virtual males water conditioned either by a familiar brother or an unrelated unfamiliar male, both owning a nest for at least 2 days, was added. Males that showed signs of breeding colouration in their holding tanks were isolated in separate tanks ($40.5 \times 20.5 \times 25$ cm). Each tank was equipped with an airstone, 2 g of dark-green wool for nest-building (Schachenmayr nomotta, polyacryl, colour no. 0072) cut into pieces of 30 ± 10 mm, and a Petri dish (diameter 9 cm) filled with washed sand. The tanks were isolated from each other with grey, opaque plastic partitions to avoid visual contact between the males. There was no significant difference between brothers and non-brothers in the time they spent separated in their holding tanks (Wilcoxon test, $n = 17$, $P > 0.05$) until they took part in a trial. Water in the holding tanks was not changed during this time. A total of 27 families were used in this study. The test males originated from 17 different families. Seven families provided a stimulus male twice, once the brother and once the unrelated male. All stimulus males and test males, however, were used only once to avoid pseudoreplication. After a male was used in a trial, the holding tank and the equipment were cleaned with a 3% solution of hydrogen peroxide and rinsed with tap water before a new male was introduced into the tank. For a detailed description of stimulus odour production, see Mehlis *et al.* (2008). The odour-conditioned water was released 3 mm under the water surface *via* a peristaltic pump (Ismatec, MS-CA4/640; www.ismatec.com) with a flow rate of 4 ml min^{-1}. This amount of odour-conditioned water was sufficient to cause an immediate reaction in receptive females in a recent mate-choice study (Mehlis *et al.*, 2008). The side at which the odour of the brother was added was alternated between trials.

A trial started by placing the test male with the nest in the experimental tank. Nests in Petri dishes that are transferred to the test aquarium are readily accepted (Rick *et al.*, 2006; Frommen *et al.*, 2009). The entry of the nest was positioned in such a way that the distance to both computer animations was equal. During an acclimatization period of 30 min after the introduction of the male, an empty landscape

was shown on the monitors (Künzler & Bakker, 1998). Then the two animations and the addition of odour were started simultaneously. Male behaviour was recorded using a webcam (Creative, Creative Webcam Live!; www.de.creative.com) that was attached 89 cm above the bottom of the tank and connected to a laptop behind the curtain. After each trial, the test tank was emptied, the whole set-up was cleaned using a 3% solution of hydrogen peroxide, rinsed with clear water and the test tank was refilled with 1 day-old tap water. This ensured that odours from previous trials would not contaminate the test water in subsequent trials (McLennan, 2004; Mehlis *et al.*, 2008).

After each trial, standard length (L_S, cm) and body mass (M, g) of all males were measured and their condition factor (K) calculated from $K = 100\ M\ L_s^{-3}$ (Bolger & Connolly, 1989). Brothers and non-brothers did not differ significantly in L_S (Wilcoxon test, $n = 17$, $P > 0.05$), M (Wilcoxon test, $n = 17$, $P > 0.05$) and K (paired t tests, $n = 17$, $P > 0.05$).

Male behaviour was analysed for 30 min. The analysis was subdivided in six blocks lasting 5 min, because a previous study showed temporal changes in response towards computer animations. The males spent much of the time near the virtual males, showing aggressive behaviour, like biting and bumping against the computer animation. As the relative time spent near a stimulus male correlates well with the relative time spent biting and bumping as well as with the mean bout length of biting-bumping time (Bakker, 1986; Rick & Bakker, 2008), the time spent in the choice zones marked 10 cm in front of each stimulus was measured (Mehlis *et al.*, 2008). Recordings started as soon as the head of the test fish had entered both choice zones (Mehlis *et al.*, 2008), which happened in all trials within 5 min after the start of the trial (mean ± S.E. 94·8 ± 21·9 s). The observer was naïve with respect to the side where the odour of the relative of the test fish was added.

Parametric statistics were used as data did not significantly deviate from normal distributions according to Kolmogorov–Smirnov tests with Lilliefors correction. For the analysis, linear mixed effect models were conducted using the R 2.4.1 statistical package (www.r-project.org/). The relative time the test male spent on each side of the test tank during the experiment was used as the dependent variable. Fixed factors were kinship (brother or non-brother) and tank side (left or right) to exclude side effects. Non-significant factors were removed from the analysis. Tests of significance were based on likelihood-ratio tests (LRT) that follow a χ^2 distribution; hence, d.f. differed by one. Furthermore, it was analysed which choice zone the test fish entered first. Test probabilities are two-tailed throughout.

All 17 males reacted vigorously to the animations with biting and bumping behaviour. The first approach of the test fish did not differ significantly between the side on which the odour of the brother or the non-kin individual was released (χ^2 test, $n_{brother} = 7$, $n_{non-kin} = 10$, $P > 0.05$). Analysing the consecutive 5 min blocks of the trials indicated that males did not spent significantly more time near the odour of the unfamiliar, non-kin male than near their brother (LRT, $n = 17$, all $P > 0.05$; Fig. 1). In addition, analysing the total 30 min revealed no significant preference for either the brother (mean ± S.E.: 51·38 ± 2·26%) or the non-kin male (48·62 ± 2·26%) (LRT, $n = 17$, $P > 0.05$). There were significant influences of the tank side in the second (LRT, $n = 17$, $P < 0.01$) and the fifth (LRT, $n = 17$, $P < 0.01$) 5 min block as well as during the total 30 min (LRT, $n = 17$, $P < 0.001$).
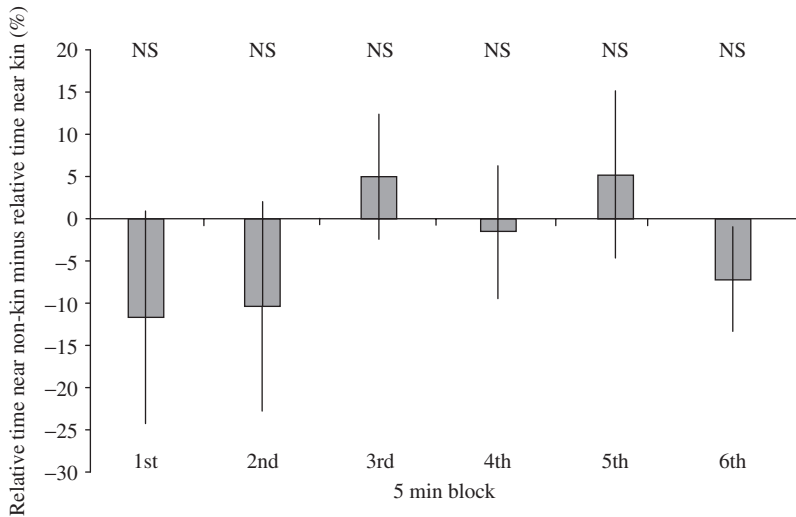
FIG. 1. Aggression preferences of male *Gasterosteus aculeatus* during successive 5 min blocks calculated as the relative time spent near non-kin minus the relative time spent near kin. Values are mean ± s.e. differences. Positive values indicate an aggression preference for the unrelated male. In all 5 min blocks, males showed no significant preference for the odour of the unfamiliar non-kin male or the odour of their brother. NS, $P > 0.05$.

The time males spent in both choice zones combined tended to decline during the 30 min, although this result failed statistical significance (Kruskal–Wallis test, d.f. = 5, $P > 0.05$; Fig. 2). Males, however, spent significantly more time in both choice zones in the first 5 min block *v.* the fifth (Mann–Whitney U-test, $n = 17$, $P < 0.05$; Fig. 2) and sixth 5 min block (Mann–Whitney U-test, $n = 17$, $P < 0.05$; Fig. 2). All other comparisons failed statistical significance (Mann–Whitney U-test, $n = 17$, all $P > 0.05$; Fig. 2).

The ability to recognize kin in different contexts, *e.g.* kin avoidance in female mate-choice (Frommen & Bakker, 2006; Mehlis *et al.*, 2008) and kin preferences in shoaling decisions (Frommen *et al.*, 2007*a*), is well documented in *G. aculeatus*, leading to the hypothesis that this ability might also be used during aggressive interactions between males. The test males, however, showed similar levels of aggression regardless whether the stimulus males were related or unrelated. Hence, the hypothesis that males might increase their inclusive fitness by exerting reduced aggressive behaviour towards their brothers was not supported.

Showing reduced aggression towards related rivals may result most likely in losing the territory, the nest or the eggs. This will lead to a decrease in direct male fitness, which may not be counterbalanced by a gain in indirect fitness. The mean coefficient of relatedness between males and their brother's offspring is 0·25, while it is 0·5 to their own offspring. Thus, to reach an increase in inclusive fitness for a male that is greater than the decrease in direct fitness, the brother has to produce twice as many offspring as a result of the reduction of the level of aggression against the brother. Therefore, it seems to be plausible that *G. aculeatus* males should defend their territory and their nest independent of the degree of kinship of a potential intruder.
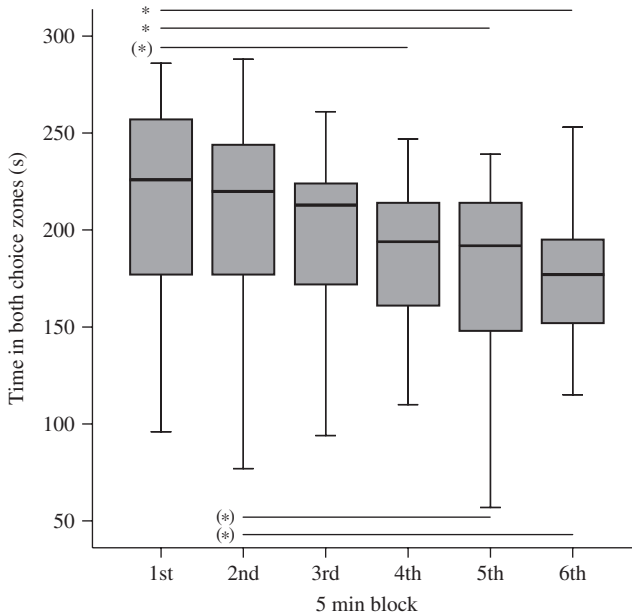
FIG. 2. The time test *Gasterosteus aculeatus* spent in both choice zones combined. The test fish spent more time attacking the computer animations at the beginning of the trial. Given are medians, quartiles and percentiles. (∗), $P > 0.01$; ∗, $P < 0.05$.

An alternative explanation for the results of the present study might be that the stimuli did not work well, either because the olfactory signal was too weak or outperformed by the visual one. In a study using the same experimental set-up, however, it was shown that female *G. aculeatus* used olfactory cues in a mate-choice context (Mehlis *et al.*, 2008). Here, females responded to the same animations within the first seconds of each trial indicating that the olfactory stimuli in the present study may have been sufficient, too. Furthermore, the males might not have recognized courting virtual males as competitors. Using the same animation, however, it was shown that males adjust their sperm expenditure when rival males were present (Zbinden *et al.*, 2003, 2004). In addition, the used animations worked well in various female mate-choice experiments (Künzler & Bakker, 1998, 2001; Mazzi *et al.*, 2003). The absence of a significant result in the present study is therefore probably not explained this way. Finally, tank-side effects might have overruled the aggression preferences for non-kin. By adding tank side as a fixed factor, however, its effect was weakened. Furthermore, a significant effect of the tank side was also found in Mehlis *et al.* (2008), but did not superimpose female's preferences for the odour of the different males.

The level of male aggression significantly decreased in the course of a trial, which could be discussed in two different ways. It could be interpreted as the result of a prolonged exposure to the stimuli (van den Assem & van der Molen, 1969; Peeke, 1969; Peeke *et al.*, 1969; Rowland, 1988). In Mehlis *et al.* (2008), females showed a significant preference for the unrelated male in the first 5 min block but this preference switched during the experiment. In the sixth 5 min block, the females significantly preferred their brother (Mehlis *et al.*, 2008). This switch might also have

been the result of the female's habituation to the frequent repeat of the same short (142 s) computer-animation sequence. Alternatively, exerting aggressive behaviour for *c.* 30 min might be very exhaustive. Males showed intensive biting and bumping towards both stimuli and this behaviour is obviously coupled with high energetic expenditure.

Summarizing, this study showed that relatedness did not significantly affect aggressive behaviour between *G. aculeatus* males. Future studies have to elucidate whether the level of aggression between relatives is reduced in a non-sexual context.

References

van den Assem, J. (1967). Territory in the three-spined stickleback *Gasterosteus aculeatus* L. An experimental study in intra-specific competition. *Behaviour (Supplement)* **16,** 1–164.

van den Assem, J. & van der Molen, J. N. (1969). Waning of the aggressive response in the three-spined stickleback after constant exposure to a conspecific. *Behaviour* **36,** 286–324.

Bakker, T. C. M. (1986). Aggressiveness in sticklebacks (*Gasterosteus aculeatus* L.): a behaviour-genetic study. *Behaviour* **98,** 1–144.

Bakker, T. C. M. (1994a). Evolution of aggressive behaviour in the threespine stickleback. In *The Evolutionary Biology of the Threespine Stickleback* (Bell, M. A. & Foster, S. A., eds), pp. 345–380. Oxford: Oxford University Press.

Bakker, T. C. M. (1994b). Genetic correlations and the control of behavior, exemplified by aggressiveness in sticklebacks. *Advances in the Study of Behavior* **23,** 135–171.

Bakker, T. C. M. & Sevenster, P. (1983). Determinants of dominance in male sticklebacks (*Gasterosteus aculeatus* L.). *Behaviour* **86,** 55–71.

Bell, M. A. & Foster, S. A. (1994). Introduction to the evolutionary biology of the threespine stickleback. In *The Evolutionary Biology of the Threespine Stickleback* (Bell, M. A. & Foster, S. A., eds), pp. 1–27. Oxford: Oxford University Press.

Bolger, T. & Connolly, P. L. (1989). The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology* **34,** 171–182.

Brown, G. E. & Brown, J. A. (1993). Social dynamics in salmonid fishes: do kin make better neighbours? *Animal Behaviour* **45,** 863–871.

Bry, C. & Gillet, C. (1980). Reduction of cannibalism in pike (*Esox lucius*) fry by isolation of full-sib families. *Reproduction Nutrition Development* **20,** 172–182.

Charlesworth, D. & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18,** 237–268.

Dzieweczynski, T. L. & Rowland, W. J. (2004). Behind closed doors: use of visual cover by courting male three-spined stickleback, *Gasterosteus aculeatus*. *Animal Behaviour* **68,** 465–471.

FitzGerald, G. J. & Morrissette, J. (1992). Kin recognition and choice of shoal mates by threespine sticklebacks. *Ethology, Ecology and Evolution* **4,** 273–283.

de Fraipont, M., Fitzgerald, G. J. & Guderley, H. (1992). Femme fatale – the case of the threespine stickleback. *Ethology* **91,** 147–152.

Frommen, J. G. & Bakker, T. C. M. (2004). Adult three-spined sticklebacks prefer to shoal with familiar kin. *Behaviour* **141,** 1401–1409.

Frommen, J. G. & Bakker, T. C. M. (2006). Inbreeding avoidance through non-random mating in sticklebacks. *Biology Letters* **2,** 232–235.

Frommen, J. G., Mehlis, M., Brendler, C. & Bakker, T. C. M. (2007*a*). Shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus*) – familiarity, kinship and inbreeding. *Behavioral Ecology and Sociobiology* **61,** 533–539.

Frommen, J. G., Brendler, C. & Bakker, T. C. M. (2007*b*). The tale of the bad stepfather: male three-spined sticklebacks (*Gasterosteus aculeatus* L.) recognize foreign eggs in their manipulated nest by egg cues alone. *Journal of Fish Biology* **70,** 1295–1301.

Frommen, J. G., Luz, C., Mazzi, D. & Bakker, T. C. M. (2008). Inbreeding depression affects fertilization success and survival but not breeding coloration in threespine sticklebacks. *Behaviour* **145,** 425–441.

Frommen, J. G., Rahn, A. K., Schroth, S. H., Waltschyk, N. & Bakker, T. C. M. (2009). Mate-choice copying when both sexes face costs of reproduction. *Evolutionary Ecology* **23**, 435–446.

Gerlach, G., Hodgins-Davis, A., MacDonald, B. & Hannah, R. C. (2007). Benefits of kin association: related and familiar zebrafish larvae (*Danio rerio*) show improved growth. *Behavioral Ecology and Sociobiology* **61,** 1765–1770.

Goldschmidt, T., Foster, S. A. & Sevenster, P. (1992). Inter-nest distance and sneaking in the three-spined stickleback. *Animal Behaviour* **44,** 793–795.

Hamilton, W. D. (1964). The genetical evolution of social behaviour I. *Journal of Theoretical Biology* **7,** 1–16.

Heckel, G., Zbinden, M., Mazzi, D., Kohler, A., Reckeweg, G., Bakker, T. C. M. & Largiadèr, C. R. (2002). Microsatellite markers for the three-spined stickleback (*Gasterosteus aculeatus* L.) and their applicability in a freshwater and an anadromous population. *Conservation Genetics* **3,** 79–81.

Jamieson, I. G. & Colgan, P. W. (1992). Sneak spawning and egg stealing by male threespine sticklebacks. *Canadian Journal of Zoology* **70,** 963–967.

Künzler, R. & Bakker, T. C. M. (1998). Computer animations as a tool in the study of mating preferences. *Behaviour* **135,** 1137–1159.

Künzler, R. & Bakker, T. C. M. (2001). Female preference for single and combined traits in computer animated stickleback males. *Behavioral Ecology* **12,** 681–685.

Largiadèr, C. R., Fries, V. & Bakker, T. C. M. (2001). Genetic analysis of sneaking and egg-thievery in a natural population of the three-spined stickleback (*Gasterosteus aculeatus* L.). *Heredity* **86,** 459–468.

Larson, G. L. (1976). Social behavior and feeding ability of two phenotypes of *Gasterosteus aculeatus* in relation to their spatial and trophic segregation in a temperate lake. *Canadian Journal of Zoology* **54,** 107–121.

Markmann, S., Hill, N., Todrank, J., Heth, G. & Blaustein, L. (2009). Differential aggressiveness between fire salamander (*Salamandra infraimmaculata*) larvae covaries with their genetic similarity. *Behavioral Ecology and Sociobiology* **63,** 1149–1155.

Mateo, J. M. (2004). Recognition systems and biological organization: the perception component of social recognition. *Annales Zoologici Fennici* **41,** 729–745.

Mazzi, D., Künzler, R. & Bakker, T. C. M. (2003). Female preference for symmetry in computer-animated three-spined sticklebacks, *Gasterosteus aculeatus*. *Behavioral Ecology and Sociobiology* **54,** 156–161.

McLennan, D. A. (2004). Male brook sticklebacks' (*Culaea inconstans*) response to olfactory cues. *Behaviour* **141,** 1411–1424.

Mehdiabadi, N. J., Jack, C. N., Farnham, T. T., Platt, T. G., Kalla, S. E., Shaulsky, G., Queller, D. C. & Strassmann, J. E. (2006). Kin preference in a social microbe – given the right circumstances, even an amoeba chooses to be altruistic towards its relatives. *Nature* **442,** 881–882.

Mehlis, M., Bakker, T. C. M. & Frommen, J. G. (2008). Smells like sib spirit: kin recognition in three-spined sticklebacks (*Gasterosteus aculeatus*) is mediated by olfactory cues. *Animal Cognition* **11,** 643–650.

Peeke, H. V. S. (1969). Habituation of conspecifics aggression in the three-spined stickleback (*Gasterosteus aculeatus* L.). *Behaviour* **35,** 137–156.

Peeke, H. V. S., Wyers, E. J. & Herz, M. J. (1969). Waning of the aggressive response to male models in the three-spined sticklebback (*Gasterosteus aculeatus* L.). *Animal Behaviour* **17,** 224–228.

Raeymaekers, J. A. M., Maes, G. E., Audenaert, E. & Volckaert, F. A. M. (2005). Detecting holocene divergence in the anadromous-freshwater three-spined stickleback (*Gasterosteus aculeatus*) system. *Molecular Ecology* **14,** 1001–1014.

Rick, I. P. & Bakker, T. C. M. (2008). Males do not see only red: UV wavelengths and male territorial aggression in the three-spined stickleback (*Gasterosteus aculeatus*). *Naturwissenschaften* **95,** 631–638.

Rick, I. P., Modarressie, R. & Bakker, T. C. M. (2006). UV wavelengths affect female mate choice in three-spined sticklebacks. *Animal Behaviour* **71,** 307–313.

Rowland, W. J. (1988). Aggression versus courtship in threespine sticklebacks and the role of habituation to neighbors. *Animal Behaviour* **36,** 348–357.

Rowland, W. J. (1989). The effects of body size, aggression and nuptial coloration on competition for territories in male threespine sticklebacks, *Gasterosteus aculeatus*. *Animal Behaviour* **37,** 282–289.

Rowland, W. J. (1994). Proximate determinants of stickleback behaviour: an evolutionary perspective. In *The Evolutionary Biology of the Threespine Stickleback* (Bell, M. A. & Foster, S. A., eds), pp. 297–344. Oxford: Oxford University Press.

Rowland, W. J., Bolyard, K. J. & Halpern, A. D. (1995). The dual effect of stickleback nuptial coloration on rivals – manipulation of a graded signal using video playback. *Animal Behaviour* **50,** 267–272.

Sargent, R. C. & Gebler, J. B. (1980). Effects of nest site concealment on hatching success, reproductive success, and paternal behavior of the threespine stickleback, *Gasterosteus aculeatus*. *Behavioral Ecology and Sociobiology* **7,** 137–142.

Thünken, T., Bakker, T. C. M., Baldauf, S. A. & Kullmann, H. (2007). Active inbreeding in a cichlid fish and its adaptive significance. *Current Biology* **17,** 225–229.

Tóth, Z., Bókony, V., Lendvai, Á. Z., Szabó, K., Pénzes, Z. & Liker, A. (2009). Kinship and aggression: do house sparrows spare their relatives. *Behavioral Ecology and Sociobiology* **63,** 1189–1196.

Vokey, J. R., Rendall, D., Tangen, J. M., Parr, L. A. & de Waal, F. B. M. (2004). Visual kin recognition and family resemblance in chimpanzees (*Pan troglodytes*). *Journal of Comparative Psychology* **118,** 194–199.

Waldman, B. & McKinnon, J. S. (1993). Inbreeding and outbreeding in fishes, amphibians and reptiles. In *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (Thornhill, N. W., ed.), pp. 250–282. Chicago, IL: University of Chicago Press.

Ward, A. J. W. & Hart, P. A. (2003). The effects of kin and familiarity on interactions between fish. *Fish and Fisheries* **4,** 348–358.

Whoriskey, F. G. & FitzGerald, G. J. (1985). Sex, cannibalism and sticklebacks. *Behavioral Ecology and Sociobiology* **18,** 15–18.

Wootton, R. J. (1984). *A Functional Biology of Sticklebacks*. London: Croom Helm.

Zbinden, M., Mazzi, D., Künzler, R., Largiadèr, C. R. & Bakker, T. C. M. (2003). Courting virtual rivals increase ejaculate size in sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology* **54,** 205–209.

Zbinden, M., Largiadèr, C. R. & Bakker, T. C. M. (2004). Body size of virtual rivals affects ejaculate size in sticklebacks. *Behavioral Ecology* **15,** 137–140.